Supplementary Materials for

Structures of Respiratory Syncytial Virus G Antigen Bound to Broadly-Neutralizing Antibodies

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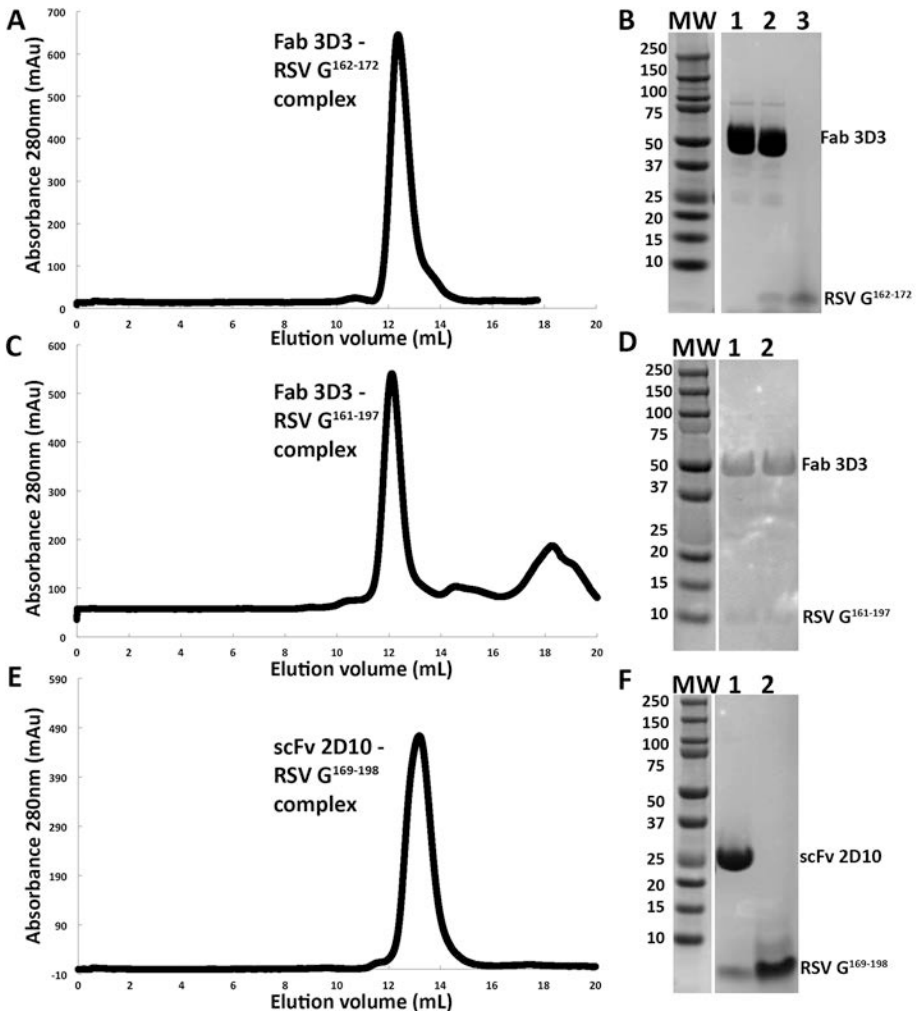


Fig. S1. Co-elution of RSV G-antibody complexes in solution. (A, C, E) Superdex 200 size-exclusion chromatography purification traces of antibody-RSV G complexes. **(B)** Non-reducing SDS-PAGE of molecular weight markers in kD (MW), Fab 3D3 alone (lane 1), co-eluted Fab 3D3-RSV $G^{162-172}$ complex (lane 2), and RSV $G^{162-172}$ alone (lane 3). **(D)** Non-reducing SDS-PAGE of molecular weight markers in kD (MW) and co-eluted Fab 3D3-RSV $G^{161-197}$ complex (lanes 1 and 2). **(F)** Reducing SDS-PAGE of molecular weight markers in kD (MW), co-eluted scFv 2D10-RSV $G^{169-198}$ complex (lane 1), and RSV $G^{169-198}$ alone (lane 2).

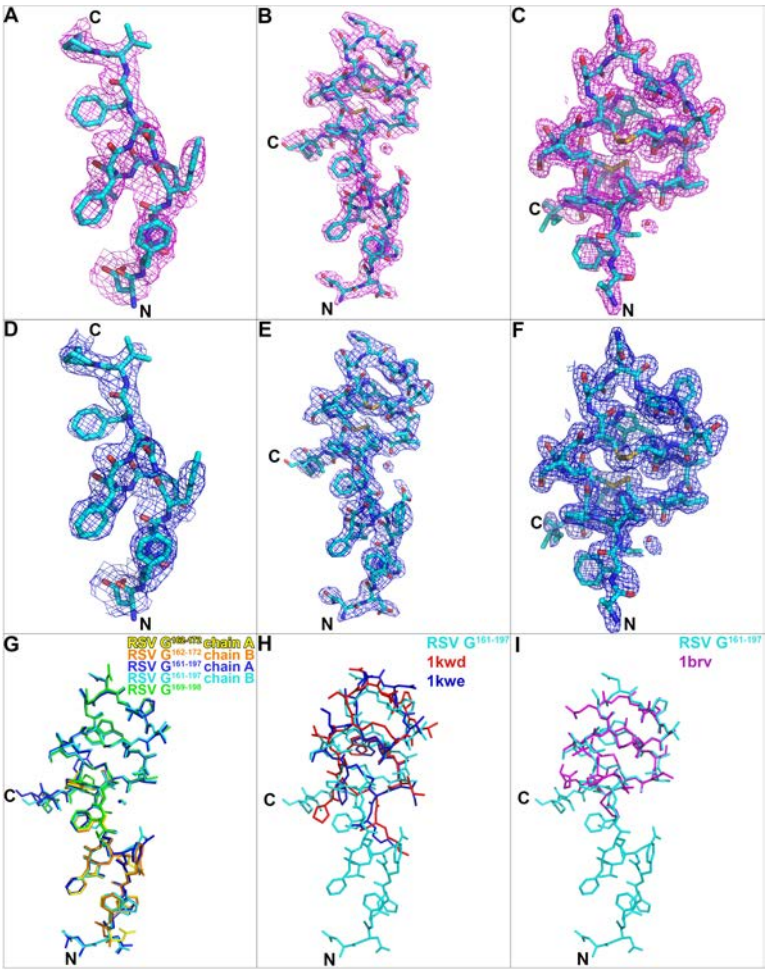


Fig. S2. Electron density maps and structural alignments of RSV G. (A, D) Electron density maps around RSV $G^{162-172}$ (when bound to Fab 3D3), **(B, E)** Electron density maps around RSV $G^{161-197}$ (when bound to Fab 3D3). **(C, F)** Electron density maps around RSV $G^{169-198}$ (when bound to scFv 2D10). The 2Fo-Fc electron density maps (in pink, panels A-C) and unbiased composite omit maps (in blue, panels D-F) are contoured at 1.0 σ. **(G)** Structural alignment of RSV G from all three structures. Note that in the Fab 3D3-RSV $G^{162-172}$ complex and Fab 3D3-RSV $G^{161-197}$ complex structures, there are two molecules of RSV G in the asymmetric unit. **(H)** Structural alignment of RSV $G^{161-197}$ with human RSV G cysteine noose structures determined previously by NMR (PDB entries 1kwd and 1kwe). The RMSD for the alignment of RSV $G^{161-197}$ with 1kwd is 1.0 Å and with 1kwe is 1.4 Å. **(I)** Structural alignment of RSV $G^{161-197}$ with bovine RSV G cysteine noose structure determined previously by NMR (PDB entries 1brv). The RMSD for the alignment of RSV $G^{161-197}$ with 1brv is 0.7 Å.

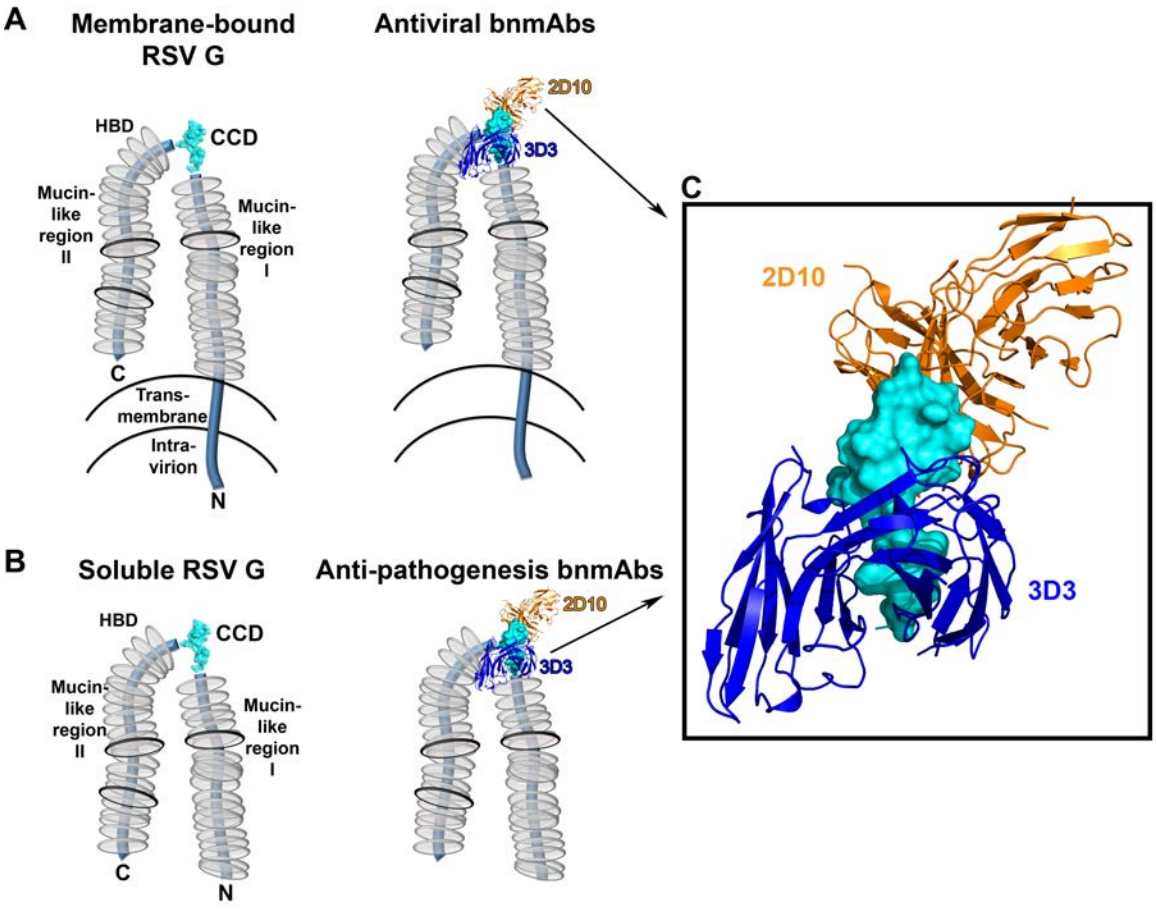


Fig. S3. Schematic of RSV G interactions with anti-G antibodies. (A) Model of membrane-bound G glycoprotein on the surface of the RSV virion or the surface of RSV-infected cells alone (left) or bound by bnmAbs 3D3 and 2D10 (right). **(B)** Model of soluble RSV G glycoprotein, which is secreted from RSV-infected cells and modulates $CX3CR1^{+}$ immune cells, alone (left) or bound by bnmAbs 3D3 and 2D10 (right). **(C)** Zoom-in of RSV G CCD bound by bnmAbs 3D3 and 2D10. For clarity, only the variable regions of the bnmAbs are shown.

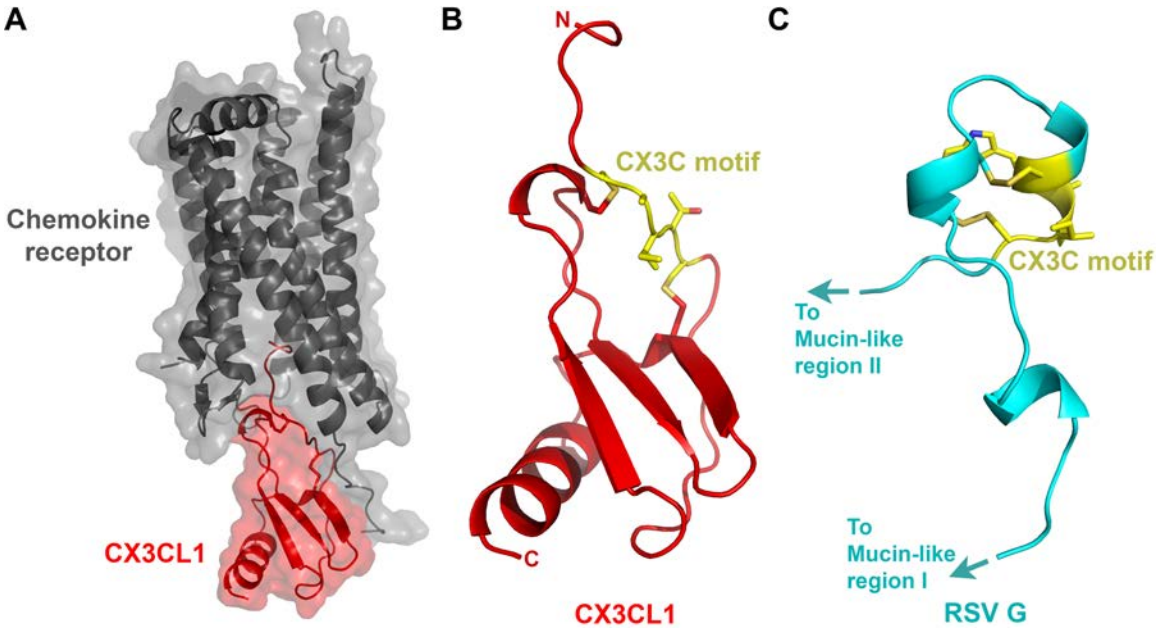
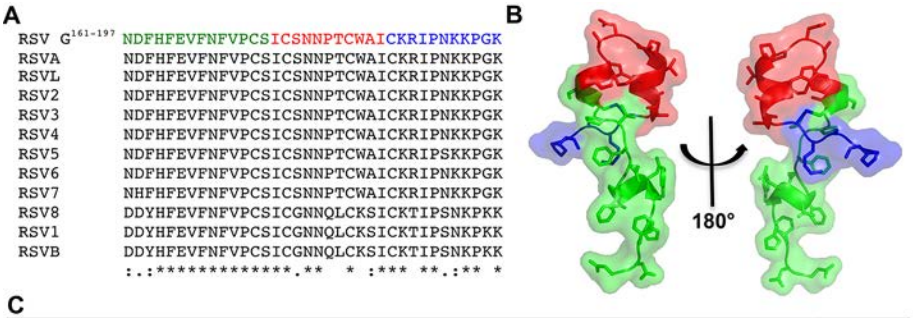


Fig. S4. Structural comparison of RSV G with CX3CL1 (fractalkine). (A) Structure of CX3CL1 (red) bound to the human cytomegalovirus G-protein coupled receptor US28 (grey) (PDB code 4XT1). US28 has 38% sequence identity with the human chemokine receptor CX3CR1. **(B)** Structure of CX3CL1 (red), in the same orientation as in panel a, with the amino acid side chains of the CX3C motif shown as sticks and colored yellow. **(C)** Structure of RSV $G^{161-197}$ (cyan)**,** with the amino acid side chains of the CX3C motif shown as sticks and colored yellow. Images in panels B and C are shown at the same magnification.



C

mAb	Linear Epitope[a]	Linear Epitope Sequence	Conformational Epitope Sequence[b]	Strain(s) Specificity	Reference[c]
3D3	164-172	----HFEVFNFVP--	NDF-FEVFNFVP-SI--N-TC--I-K-IP	RSV A, B	Collarini et al. 2009
2B11	162-172	--DFHFEVFNFVP--		RSV A, B	Collarini et al. 2009
1G8	164-172	----HFEVFNFVP--		RSV A, B	Collarini et al. 2009
10C6	165-169	-----FEVFN-----		RSV A, B	Collarini et al. 2009
1D4	165-171	-----FEVFNFV---		RSV A, B	Collarini et al. 2009
3G12	167-176	-------VFNFVPCSIC		RSV A, B	Collarini et al. 2009
1F12	166-172	------EVFNFVP--		RSV A, B	Collarini et al. 2009
1A5	161-170	-NDFHFEVFNF----		RSV A, B	Collarini et al. 2009
5D8	160-169	NNDFHFEVFN-----		RSV A, B	Collarini et al. 2009
2D10	N.D.	N.D.	C---SNNPT-WAICKR	RSV A, B	Collarini et al. 2009
1G1	N.D.	N.D.		RSV A, B	Collarini et al. 2009
6A12	N.D.	N.D.		RSV A, B	Collarini et al. 2009
AT34	161-170	-NDFHFEVFNF----		RSV A, B	Cortjens et al. 2017
AT40	162-170	--DFHFEVFNF----		RSV A, B	Cortjens et al. 2017
AT64G	162-170	--DFHFEVFNF----		RSV A, B	Cortjens et al. 2017
AT50	175-184	-ICSNNPTCWA-		RSV A	Cortjens et al. 2017
AT51	174-184	SICSNNPTCWA-		RSV A	Cortjens et al. 2017
AT61G	174-184	SICSNNPTCWA-		RSV A	Cortjens et al. 2017
AT32	185-193	ICKRIPNKK---		RSV A	Cortjens et al. 2017
AT33	186-193	-CKRIPNKK---		RSV A	Cortjens et al. 2017
AT42	N.D.	N.D.		RSV A, B	Cortjens et al. 2017

[a] Linear epitope sequence numbering in RSV strain A2. N.D. refers to no detectable binding to linear RSV G peptides.
[b] Conformational epitope sequences were determined in this study.
[c] References for linear epitope sequence mapping.

Fig. S5. Isolated human anti-G mAbs and epitope characterization. (A) Sequence alignment of RSV G CCD from diverse RSV strains, colored by linear epitope clustering in three regions. **(B)** Structure of RSV $G^{161-197}$, colored as in panel a, revealing the overlap of linear epitopes in three-dimensional space. **(C)** Table of 21 isolated human anti-G mAbs, their linear epitope sequences, and their RSV strain specificity.

Table S1. Crystallographic data collection and refinement statistics[a]

	3D3-RSV $G^{162-172}$	3D3-RSV $G^{161-197}$	2D10-RSV $G^{169-198}$
PDB code	5WNB	5WNA	5WN9
Data collection[b]			
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
Cell dimensions			
a, *b*, *c* (Å)	68.76, 105.43, 121.82	64.62, 135.01, 73.78	44.84, 56.39, 126.15
α, β, γ (°)	90, 90, 90	90, 107.45, 90	90, 90, 90
Resolution (Å)	48.38 - 2.40 (2.48 – 2.40)	48.72 - 2.40 (2.48 - 2.40)	50.00 - 1.55 (1.58 – 1.55)
Total no. reflections	368,399	294,679	453,130
No. unique reflections	40,103	46,906	47,198
R_{merge}[c]	0.122 (0.838)	0.107 (0.763)	0.058 (0.930)
I / σ(I)	13.4 (3.1)	12.3 (2.2)	44.0 (1.4)
Completeness (%)	99.9 (99.8)	99.5 (99.0)	99.9 (99.4)
Redundancy	9.2 (8.3)	6.3 (5.6)	9.6 (6.2)
$CC_{1/2}$[d]	0.997 (0.808)	0.996 (0.670)	0.998 (0.751)
Refinement			
Resolution (Å)	48.38 - 2.40	48.72 - 2.40	50.00 - 1.55
No. reflections	35,325	46,869	47,114
R_{work} / R_{free}[e]	0.224 / 0.267	0.192 / 0.246	0.169 / 0.185
No. atoms			
Protein	6,552	7,124	3,810
Ligand/ion	22	None	None
Water	90	135	111
B-factors (Å^2)			
Protein: bnmAb	50	41	38
Protein: RSV G	40	56	48
Ligand/ion	63	None	None
Water	35	39	38
R.m.s. deviations			
Bond lengths (Å)	0.005	0.006	0.008
Bond angles (°)	0.864	0.935	0.968
Ramachandran (%)			
Favored	96.5	97.6	99.2
Allowed	3.5	2.4	0.8
Outliers	0	0	0

[a] For each structure, data from one crystal was used.

[b] Values in parentheses are for highest-resolution shell.

[c] $R_{merge} = \Sigma|(I - <I>)| / \Sigma(I)$, where I is the observed intensity.

[d] $CC_{1/2}$ = Pearson correlation coefficient between random half-datasets.

[e] $R_{work} = \Sigma||F_o| - |F_c|| / \Sigma|F_o|$ for all data except 5%, which were used for R_{free} calculation.